(UNDATED)

DATA EVALUATION RECORD

STUDY 4

CHEM 112600 Prohexadione calcium §162-1
CAS No. 127277-53-6
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44457785
Venkatesh, K. 1996. Aerobic soil metabolism of ^{14}C-BAS 125 W (9054 W). BASF Report No.: M9417. BASF Reg. Document No.: 96/5001. Unpublished study performed by BASF Corporation, Research Triangle Park, NC, and AGVISE Laboratories, Northwood, ND, and submitted by BASF Corporation, Research Triangle Park, NC.

DIRECT REVIEW TIME = 47 Hours

REVIEWED BY:	D. R. Hunt, B.A.	Signature:
TITLE:	Scientist	Date:
EDITED BY:	C. A. Little, Ph.D.	Signature:
TITLE:	Sr. Scientist/Asst. Project Manager	Date:
APPROVED BY:	P. H. Howard, Ph.D.	Signature:
TITLE:	Project Manager	Date:
ORG:	Syracuse Research Corp. Arlington, VA 22202	
TEL:	703/413-9369	

APPROVED BY:	Iwona L. Maher, Chemist	James A. Hetrick, Ph.D., Chemist
ORG:	ERB I/EFED/OPP	ERB I/EFED/OPP
TEL:	703/605-0569	703/305-5237
DATE:	Iwona L. Maher	James A. Hetrick

SIGNATURE:

CONCLUSIONS

Metabolism - Aerobic Soil

1. This study is scientifically valid and satisfies the Subdivision N data requirement for the aerobic soil metabolism.

2. Radiolabeled ^{14}C-prohexadione calcium at a nominal application rate of 0.13 ppm degraded with the EFED calculated, nonlinear first order kinetics, half-life ($T_{1/2}$) of 1.4 days ($r^2 = 0.98$; a linear first order kinetics $T_{1/2}$ was 9.8 days with $r^2 = 0.73$), in sandy loam soil approximately at 80% of 0.33 bar moisture content and incubated in darkness at 25 ± 1 °C.

3. The main degradate is CO_2 which accounted for the maximum of 79.8% of the applied radioactivity at 30 days posttreatment. The minor degradate, 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid, was a maximum of 2.2% (0.003 ppm) at 0.5 days posttreatment. An unidentified minor degradate was present at a maximum of 1.2% (0.002 ppm) of the applied radioactivity at 3 days posttreatment.

METHODOLOGY

Samples (25 g) of sieved (2 mm) sandy loam soil (collected from Field Test Site in Holy Springs, NC; 78% sand, 11% silt, 11% clay, pH 6.0, 0.7% organic matter, CEC 4.7 meq/100 g; Table 2, p. 32) at approximately 80% of the moisture content at 0.33 bar (6.37% - initial soil moisture) were weighed into Erlenmeyer flasks and treated dropwise by syringe with cyclohexene-ring labeled [3,5-^{14}C]prohexadione calcium (BAS 125 W, calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate; radiochemical purity 98.6%, specific activity 15.3 mCi/mmol, pp. 9, 10), dissolved in acetonitrile and 1 *N* HCl (volume ratio unspecified), at a nominal application rate of 0.13 ppm (pp. 11, 12). Flasks containing treated soil samples were mixed by swirling, and incubated in darkness at 25 ± 1 °C for up to 62 days (Figure 3, p. 52). To capture $^{14}CO_2$, CO_2-free, moist air was pumped through the flasks and into two 2 *N* NaOH traps (p. 13). The soil samples were treated at different dates over a two-month period (Table 3, p. 33) and individual samples were removed for analysis at 0, 0.5, 1, 2, 3, 5, 7, 14, 30, and 62 days posttreatment.

At each sampling interval, soil samples were immediately extracted twice with acetone:1 *N* H_2SO_4 (12:4, v:v), followed twice with acetone, and once with an acetone Soxhlet extraction. The combined extracts were analyzed for total radioactivity by LSC (p. 15; Figure 5, p. 54). An aliquot of the combined extracts was partitioned three times with dichloromethane:methanol (3:1, v:v), and the total radioactivity in the organic and aqueous phases was determined by LSC. The organic phase was analyzed by reverse-phase HPLC (Hamilton C-18 PRP-1 column) using a mobile phase gradient of 0.01% acetic acid in water:acetonitrile (95:5 to 10:90, v:v) with

radioactive flow detection and fraction collection followed by analysis of fractions for total radioactivity by LSC (p. 17). To confirm the compound identity of the parent, the organic phase was further analyzed by TLC using silica gel plates developed in isopropyl ether:formic acid:water (90:7:3, v:v:v); samples were co-chromatographed with reference standards and [^{14}C]residues were determined by radioimage scanning (pp. 16, 17). To confirm the identity of the degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid, the organic fraction from 5 days posttreatment soil was analyzed by HPLC (YMC-OD Aq column) using a mobile phase gradient of acetonitrile plus 0.1% trifluoroacetic acid:water plus 0.1% trifluoroacetic acid (5:95 to 100:0, v:v; pp. 17, 18); the sample was co-chromatographed with nonradiolabeled reference standards of the parent and degradate (p. 23; Figure 14, p. 63). TLC and HPLC analyses of the final organic phases were completed within 7 days of extraction (Table 3, p. 33). Following extraction, soil subsamples were analyzed for total radioactivity by LSC following combustion (p. 15).

Post-extracted soils from 2, 7, 14, and 30 days posttreatment were further analyzed to determine radioactivity associated with the organic matter (Figure 6, p. 55). Samples (10-20 g) of post-extracted soil were refluxed with 100 mL of 0.1 *N* NaOH for 2 hours, centrifuged, and the supernatant decanted (p. 15). Total radioactivity remaining in the soil was determined by LSC following combustion. The supernatant was partitioned into aqueous and organic phases using dichloromethane. Total radioactivity in the organic phase was determined by LSC (p. 16). The aqueous phase was adjusted to pH 1.0 with HCl and centrifuged, and the supernatant was decanted; the precipitate (humic acid) was dissolved in 0.5 M NaOH and the radioactivity was determined by LSC. The supernatant was partitioned into organic (fulvic acid) and aqueous phases with ethyl acetate; radioactivity in each phase was determined by LSC. A selected sample (1 day posttreatment) of the organic phase extracts was analyzed by LC/MS (Primesphere C18 - HC column) using an isocratic mobile phase of MeOH/H_2O:4 mM ammonium formate/1% formic acid with radioactive flow detection and mass selective detection in the negative ionspray mode (p. 18).

Trapped $^{14}CO_2$ was released by treatment with 5 *N* H_2SO_4 and collected in a scintillation cocktail; total radioactivity was determined by LSC (p. 18). The presence of $^{14}CO_2$ was confirmed by precipitation with $BaCl_2$.

A frozen storage stability study was performed to determine the stability of prohexadione calcium in soil and soil extracts (pp. 13-15; Figure 4, p. 53). A soil sample (300 g) fortified with prohexadione calcium at a nominal application rate of 0.125 ppm, was divided in two. The first half of the sample was incubated as described above for up to 21 days. Subsamples were collected at 2 and 21 days posttreatment and stored frozen for 25 and 40 days. Following frozen storage, the subsamples were analyzed for total radioactivity by LSC following combustion and by HPLC and TLC as described previously. The second half of the treated sample was immediately frozen (-20 ± 1 °C) and stored for up to 183 days. Subsamples were removed for analysis at 0, 12, 27, 138, and 183 days posttreatment. At each sampling interval, subsamples were analyzed for total radioactivity by LSC following combustion. Additional subsamples were

extracted and analyzed by HPLC and TLC as described previously; soil extracts were analyzed following 0, 12 and 27 days of frozen storage. Recoveries of prohexadione calcium from fortified soil samples were 79.9-86.3% of the applied radioactivity following 0-183 days of frozen storage with no clear pattern of degradation (Table 14, p. 44). Recoveries of the parent from the soil extract and organic phase of the soil extract were 86.3-93.6% and 86.1-86.3% of the applied, respectively, following 0-27 days of frozen storage (Tables 15, 16; pp. 45, 46). In soil treated with the parent and incubated for 2 days, the parent was initially present at 46.0% of the applied radioactivity following incubation. Following incubation and 40 days of frozen storage, the parent was present at 45.8% of the applied (Table 19, p. 49).

STUDY AUTHOR'S DATA SUMMARY

Cyclohexene-ring labeled [3,5-^{14}C]prohexadione calcium (radiochemical purity 98.6%), at a nominal application rate of 0.13 ppm, degraded with a registrant-calculated DT_{50} of 1 day (determined through a nonlinear model, $r^2 = 0.98$) in sandy loam soil at approximately 80% of 0.33 bar moisture content and incubated in darkness at 25 ± 1 °C for up to 62 days (p. 24; Table 12, p. 42; Figure 18, p. 68). Data reported as percentages of the applied represent percentages of the nominal application; degradate concentrations are reported in parent equivalents. Based on HPLC analysis, the parent compound was initially present at 91.7% (0.12 ppm) of the applied radioactivity. It decreased to 50.8% (0.066 ppm) by 1 day and 26.9% (0.035 ppm) by 2 days posttreatment, and was 1.1-1.2% (0.001-0.002 ppm) from 30 to 62 days posttreatment (Table 8, p. 38). The minor degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid was initially 1.1% (0.001 ppm; day 0) of the applied radioactivity, increased to a maximum of 2.2% (0.003 ppm) by 0.5 days posttreatment, and was ≤0.001 ppm from 2 to 62 days. An unidentified minor degradate was first present at a maximum of 1.2% (0.002 ppm) of the applied radioactivity at 3 days posttreatment and was ≤0.001 ppm from 5 to 62 days. Nonextractable [^{14}C]residues were 4.0% of the applied radioactivity at 0.5 days posttreatment, increased to 14.6% by 5 days, and were 10.6% at 62 days posttreatment (Table 6, p. 36); radioactivity associated with the humic acid and fulvic acid fractions from 2-30 days posttreatment was 3.2-4.5% and 1.4-3.0% of the applied radioactivity, respectively (Table 11, p. 41). Evolved $^{14}CO_2$ was initially 12.9% of the applied radioactivity at 0.5 days posttreatment, increased to 51.4% by 2 days and decreased from 79.8 to 77.7% from the 30th day to the 62nd day posttreatment (Table 10, p. 40).

The HPLC peak labeled "#1" (Table 8, p. 38) was 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (Table 1, p. 31). Identification of peak #1 was confirmed by co-chromatography during HPLC analysis of the organic fraction from 5 days posttreatment soil with nonradiolabeled reference standards (p. 23; Figure 14, p. 63).

Material balances (based on LSC analysis) were 93.9-98.8% of the applied radioactivity throughout the incubation period (Table 6, p. 36).

THE REVIEWERS' COMMENTS

1. In the sandy loam soil (78% sand, 11% silt, 11% clay, pH 6.0, 0.7% organic matter, CEC 4.7 meq/100 g) prohexadione calcium biodegraded aerobically with the EFED calculated, nonlinear first order kinetics, $T_{1/2}$ of 1.4 days (derived from $C = a*e^{-kt}$ where $T_{1/2} = (\ln 2)/k = 0.693/k$; k - a slope of the regression line; $r^2 = 0.98$) and linear $T_{1/2}$ of 9.8 days ($\ln C_t = \ln C_0 - kt$; $r^2 = 0.73$). Both linear and nonlinear regressions were performed to calculate the degradation half-lives because soil aerobic degradation data presented a biphasic distribution. In the nonlinear regression some data points were outweighed compared to others. Thus, a higher r^2 was the chosen criterion for half-life selection between the two obtained values (e.g., for the modeling purposes aimed in the estimates of surface and ground water concentrations).

   The minor degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid was a maximum of 2.2% (0.003 ppm) at 0.5 days posttreatment. Nonextractable [^{14}C]residues were a maximum of 14.6% at 5 days, and were 10.6% at 62 days posttreatment; radioactivity associated with the humic acid and fulvic acid fractions from 2 to 30 days posttreatment was 3.2-4.5% and 1.4-3.0% of the applied, respectively. Radiolabeled $^{14}CO_2$ was 12.9% of the applied radioactivity at 0.5 days posttreatment, increased to 51.4% by 2 days and dropped from 79.8 to 77.7% from the 30th to the 62nd day.

2. To ensure aerobic conditions and soil viability aerobic soil metabolism studies should be performed at 75% of the soil moisture at 0.33 bar. It could not be determined whether the soil moisture content in this study was maintained at 75% of 0.33 bar throughout the incubation period. Initial moisture of the soil was 6.37%, equivalent to 80% at 1/3 bar, and during the test moist air was continuously purged through the sample through negative air flow.

3. The microbial biomass may not be consistent during the test since the soil samples were stored and treated at different dates over a two-month period and then incubated for up to 62 days (Table 3, p. 33). At each sampling interval only single replicates were used to determine the aerobic soil degradation of the test compound.

4. Method detection limits and limits of quantitation were not reported for LSC, HPLC or TLC.

REFERENCES

Cowlyn, T.C. "BA-112: Determination of Physico-Chemical Properties: Part J: Determination of Water Solubility". LSR Report No. 91/KCI128/0748, BASF Registration Document No. 93/11530, 7/04/01.

Prohexadione Calcium

DER MRID 444577-85

Page____ is not included in this copy.

Pages 6 through 29 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
